Journal of Neurochemistry
Raven Press, New York

A Simple and Rapid Method for the Purification of Ovine Pineal Arylalkylamine *N*-Acetyltransferase

M. A. A. Namboodiri, *M. J. Brownstein, Pierre Voisin, Joan L. Weller, and David C. Klein

*Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, and *Laboratory of Cell Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland, U.S.A.*

Abstract: A two-step chromatographic procedure has been developed for the purification of ovine pineal arylalkylamine *N*-acetyltransferase (EC 2.3.1.87), based on the principles of disulfide exchange and anion exchange. The enzyme from 20 ovine pineal glands can be purified about 500-fold in a day; recovery is about 5%. Polyacrylamide gel electrophoretic analysis of the final preparation shows four major bands; one appears to be arylalkylamine *N*-acetyltransferase. **Key Words:** Cystamine—Disulfide exchange—Fast protein liquid chromatography—Anion exchange.
Namboodiri M. A. A. et al. A simple and rapid method for the purification of ovine pineal arylalkylamine *N*-acetyltransferase. *J. Neurochem.* **48,** 580–585 (1987).

Arylalkylamine *N*-acetyltransferase (EC 2.3.1.87, serotonin *N*-acetyltransferase, NAT) converts serotonin to *N*-acetylserotonin, the precursor of melatonin (*N*-acetyl-5-methoxytryptamine), the pineal hormone (Weissbach et al., 1960). This enzyme is of special interest because there is a large precisely controlled daily rhythm in its activity (Axelrod and Zatz, 1977; Klein et al., 1981). In the rat, activity increases at night in the dark with a doubling time of about 15–20 min to values that are about 30- to 70-fold greater than day values. This increase involves neurally controlled induction and activation mechanisms (Romero et al., 1975; Klein, 1978). When animals are exposed to light during the night, neural stimulation is terminated, and activity of the enzyme decreases rapidly ($t_{1/2}$ = 3–4 min) (Klein and Weller, 1972). This appears to be due to inactivation of the enzyme, possibly involving regulatory disulfide peptides (Binkley et al., 1976; Namboodiri et al., 1980, 1981). It is generally believed that the activity of this enzyme regulates large and rapid changes in pineal melatonin synthesis, pineal melatonin, and blood melatonin (Reppert and Klein, 1980).

Purification of pineal NAT has been difficult because the tissue is small and the enzyme is an unstable minor protein. In a preliminary report we have described a procedure for partially purifying ovine pineal NAT (Namboodiri and Klein, 1981), which exhibits a fivefold nocturnal increase in activity, dependent on protein synthesis (Namboodiri et al., 1985*a,b*). We have concentrated our efforts on the ovine enzyme because the ovine gland is larger. In our previous procedure the final preparation contained relatively large amounts of bovine serum albumin (BSA) and associated protein contaminants because it was necessary to use BSA as a carrier to stabilize the enzyme. This precluded a number of analyses, including protein sequencing. We now describe a rapid and simple procedure that purifies the enzyme about 500-fold, without BSA.

MATERIALS AND METHODS

Materials

Pineal glands were obtained from adult male and female (Dorsett × Rambouillet cross) sheep kept in a controlled lighting environment (L/D 12:12) for at least 5 days at the NIH Animal Center. The dark period started at 1500 h and animals were killed between 2000 and 2100 h in the dark by injection of a lethal dose of Somlethal. Within 5 min after

Received March 31, 1986; revised June 12, 1986; accepted August 14, 1986.

Address correspondence and reprint requests to Dr. D. C. Klein at Building 10, Room 8D-42C, National Institutes of Health, Bethesda, MD 20892, U.S.A.

The present address of Dr. M. A. A. Namboodiri is Department of Biology, Georgetown University, Washington, D.C. 20057, U.S.A.

Abbreviations used: BSA, bovine serum albumin; DTT, dithiothreitol; $NaDodSO_4$-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NAT, arylalkylamine *N*-acetyltransferase; TCA, trichloroacetic acid.

death, the pineal gland had been removed and placed on solid CO_2; glands were stored at −70°C for up to 4 months.

To prepare Sepharose-cystamine, cyanogen bromide-activated Sepharose (25 g) is added to 100 ml of 0.1 *M* sodium acetate, pH 6.0, containing cystamine-HCl (2.25 g); the suspension is gently stirred for 6 h (20°C) (Chibata et al., 1974). The resulting Sepharose-cystamine is filtered, washed sequentially with 100 ml of 1 *M* sodium chloride and 500 ml of the sodium acetate buffer, and then stored (0–2°C) in this buffer. The resulting Sepharose-cystamine functions consistently for a period of 6 months.

A fast protein liquid chromatography column (MonoQ) was obtained from Pharmacia Fine Chemicals and Bolton-Hunter reagent from New England Nuclear Corporation; other reagents, chemicals, and supplies were obtained from commercial sources.

Preparation of homogenates

Each gland (~100 mg) is minced and homogenized (0–2°C) in 10 m*M* ammonium acetate, pH 6.5 (1 mg/10 μl), using a Potter-Elvehjem homogenizer (0–4°C). The homogenate is centrifuged (10,000 *g*, 30 min, 0–2°C) and the supernatant is used immediately.

NAT assay

A modification (Parfitt et al., 1975) of the Deguchi and Axelrod assay is used (Deguchi and Axelrod, 1972). A 50-μl aliquot of 100 m*M* sodium phosphate buffer, pH 6.8, containing 1-[^{14}C]acetyl-CoA (0.24 m*M*; sp act 8.3 Ci/mol) and tryptamine (20 m*M*) is incubated (37°C; 30 min) with a 50-μl sample. The reaction is terminated by the addition of 1 ml of chloroform, which extracts the product *N*-[^{14}C]acetyltryptamine. The aqueous layer is removed, and the chloroform is washed once with 0.2 ml of 100 m*M* sodium phosphate buffer, pH 6.8, and twice with 0.2 ml of 1 *M* NaOH. A 0.5-ml sample of chloroform is taken to dryness, and radioactivity is measured by routine methods.

Disulfide-exchange chromatography

A chromatographic column (1 × 5 cm) containing Sepharose-cystamine is equilibrated (0–4°C) with 10 m*M* veronal-HCl buffer, pH 8.5, containing 100 m*M* sodium citrate. A 20-ml sample of the supernatant is mixed with 40 ml of 30 m*M* veronal-HCl buffer, pH 8.5, containing 300 m*M* sodium citrate; the column is loaded (50 ml/h) with this solution. The effluent is reapplied to the column to maximize binding of the enzyme. The column is then washed (100 ml/h) sequentially with 20 ml of the equilibrating buffer; 40 ml of 100 m*M* sodium citrate, pH 6.5; and finally, with 40 ml of 100 m*M* ammonium acetate, pH 6.5, containing 10 m*M* dithiothreitol (DTT). The flow is then stopped for 8–12 h to allow reduction of the mixed disulfide formed between the proteins and Sepharose-cystamine to go to completion. Flow is resumed with the same buffer (about 50 ml) until no protein is detectable in the eluate. Enzyme activity is eluted using 100 m*M* Tris HCl, pH 8.5, containing 1 m*M* DTT; 90% the eluted enzyme activity is usually found in the first 20 ml.

Anion-exchange chromatography

A MonoQ column is equilibrated (0–4°C) with Tris HCl (20 m*M*, pH 8.4) containing glycerol (10%) using a Gilson HPLC system. A 10-ml sample of the pooled fractions from the previous step is diluted to 40 ml using deionized water and loaded into the column by repeated injections using a 1-ml loop (flow rate, 1 ml/min). The column is then washed using the equilibrating buffer (10 ml) and the enzyme activity is eluted using a linear gradient of NaCl (0–1.0 *M*, 30 min, 1 ml/min) in Tris HCl (20 m*M*, pH 8.4), containing glycerol (10%), and the 1.0 *M* NaCl containing DTT (10 m*M*). Fractions (~4 ml) of the effluent from the loading step and fractions (~1 ml) of the wash and gradient are collected, transferred immediately onto wet ice, and then assayed for NAT activity. This step takes about 1 h.

Precipitation of proteins using trichloroacetic acid (TCA)

A 250-μl sample of TCA (50%) is added to a 1-ml protein sample containing sodium deoxycholate (0.1%). After 1 h at 0–2°C, the sample is centrifuged (10,000 *g*, 10 min); the supernatant is removed and the pellet is washed twice with acetone (1 ml).

Radioiodination of proteins using ^{125}I-Bolton-Hunter reagent

Proteins are labeled using the Bolton-Hunter reagent as described (Bolton and Hunter, 1973) with the following modifications. A TCA-precipitated protein sample is dissolved in 25 μl of 0.1 *M* borate buffer, pH 8.5, added to a "Combi-u-vial" containing the air-dried iodinated ester, agitated, and kept at 0°C for 18 h to complete the reaction. The proteins are then precipitated using TCA as described above and prepared for electrophoresis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis ($NaDodSO_4$-PAGE) analysis

The samples of crude or purified sheep NAT are precipitated with TCA and solubilized (90°C, 5 min) in 40 μl of sample solution (2.3% $NaDodSO_4$, 5% β-mercaptoethanol, 10% glycerol, 60 m*M* Tris HCl, pH 6.8). The samples are applied on a 15% acrylamide, 0.1% $NaDodSO_4$ slab gel with a 3% acrylamide stacking gel and electrophoresis is run at 20 mA constant current (Laemmli, 1970). The gels are fixed in 50% methanol containing 10% acetic acid overnight. Silver staining is performed by a modification of a procedure described by Morrissey (1981) with successive incubation in 5% glutaraldehyde (30 min), 0.1 m*M* DTT (30 min), 0.2% silver nitrate (30 min), and 3% sodium carbonate containing 0.02% formaldehyde (3–10 min). Commassie Brilliant Blue-R staining is performed by a standard procedure (Sarkar and Dion, 1975) in 45% methanol containing 10% acetic acid. When autoradiography is required, the gels are dried and exposed to a Kodak XAR film between two intensifying screens for 2–24 h.

Protein measurement

Proteins are estimated with a micro dye binding procedure in 96-well micro titer plates (Bradford, 1976), using an automatic ELISA reader (Micromedic). BSA is used as a standard.

RESULTS

Purification

Near-complete binding of enzyme activity on the column occurs during loading in disulfide-exchange chromatography (Table 1). No significant amount of the enzyme, either in the active form or inactive form as detected by its capacity to be reactivated by DTT

TABLE 1. *Purification of NAT using a Sepharose-cystamine column*

Chromatographic fractions	Total enzyme activity (nmol/min)	Percent load	Total protein (mg)	Percent load	Sp act (nmol/min/mg protein)	-Fold purification
Load	31.1	100	120	100	0.26	—
Effluent	0.6	2	97.5	81	0.006	—
DTT wash	3.1	10	2.8	2	1.1	4.2
pH eluate	17.5	56	3.2	3	5.5	21

A 20-ml sample of pineal gland supernatant was mixed with 40 ml of 30 m*M* veronal HCl buffer, pH 8.5, containing 300 m*M* sodium citrate, and the column was loaded with this solution. The column was washed sequentially with 20 ml of the equilibrating buffer; 40 ml of sodium citrate, pH 6.5; and finally with 40 ml of 100 m*M* ammonium acetate containing DTT (10 m*M*). The flow was then stopped for 8–12 h and resumed with the same buffer until no protein was detectable in the eluate. NAT activity was then eluted using Tris HCl pH 8.5, containing DTT (1 m*M*). NAT activity and proteins were measured in 50-μl aliquots. Values of activity and proteins for the fractions from the loading step and the first wash step using the equilibrating buffer are given under "Effluent." No proteins could be detected in the sodium citrate, pH 6.5 wash and the first ammonium acetate-DTT wash; the values for the second ammonium acetate-DTT wash are given under "DTT wash."

treatment, is found in the effluent. Small amounts of the enzyme activity (10%) and proteins (2%) are released after reduction of the mixed disulfide by DTT. Washing the column using 100 m*M* Tris HCl, pH 8.4, containing DTT (1 m*M*) elutes about 60% of the enzyme activity loaded on the column. This step provides a 21-fold purification.

Almost all of the proteins including the enzyme bind to the anion-exchange column under the conditions of loading (Fig. 1); no enzyme activity or proteins is detected either in the effluent or the wash. NAT activity is eluted at the start of the gradient (20–100 m*M* NaCl) whereas the majority of the proteins are eluted in the later fractions. This step yields about

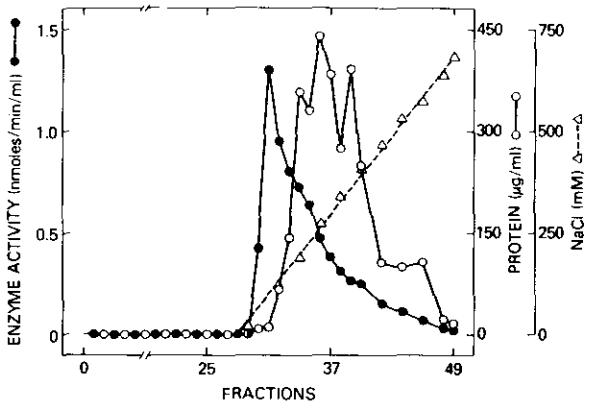

FIG. 1. Purification of NAT by anion-exchange chromatography using MonoQ column. A 10-ml sample of the pooled fractions from the Sepharose-cystamine chromatographic step was diluted to 40 ml using deionized water and loaded onto the column. The column was then washed using the equilibrating buffer (10 ml), and the enzyme activity was eluted using a linear gradient of NaCl in Tris HCl (20 m*M*, pH 8.4) containing glycerol (10%) and DTT (10 m*M*). Fractions were collected and were transferred immediately onto wet ice. Enzyme activity and proteins were measured in aliquots of 100 μl. Concentration of NaCl was estimated by measuring conductivity, using a Markson Portable Conductivity Meter.

40% recovery of activity [the recovery is only about 10% when enzyme activity in the purest fraction (31) alone is used for calculation].

The entire purification procedure requires about 24 h, and yields a preparation of NAT with about 500-fold purification and a net recovery of activity of about 5% (Table 2).

Analysis

The protein patterns obtained after NaDodSO$_4$-PAGE at the sequential steps in the purification procedure from a typical experiment are presented. Using Commassie Blue Stain, the fractions obtained after the disulfide-exchange step showed an enrichment of proteins at M_r = 50,000, 40,000, and 20,000 as compared to the crude supernatant and retained a faintly stained protein band at M_r = 12,000 (Fig. 2, lane B). Protein bands could not be detected in the MonoQ fraction of highest purity using the Commassie Blue stain. However, using a silver stain procedure this fraction showed protein components at M_r = 12,000, 20,000, 40,000, and 65,000 in one out of three experiments (Fig. 2, lane C). The more sensitive method of detection by radiolabeling with the ^{125}I-Bolton-Hunter reagent was used. Analysis of the fractions of highest purity obtained in three separate experiments revealed that four protein bands were detected with this procedure (Fig. 2, lane D). One intensely labeled protein band (M_r = 12,000) was observed in all experiments. Protein components of variable intensities were observed at M_r = 20,000, 40,000, and 65,000.

DISCUSSION

In the present article we have described a simple and rapid procedure for the purification of ovine pineal NAT. It is of interest to compare the present method with the previously reported one to understand its advantages. The previous procedure involved disulfide-exchange chromatography using

TABLE 2. *Purification of NAT from pineal glands*

Purification step	Total protein (mg)	Total activity (nmol/min)	Sp act (nmol/min/mg protein)	Yield (%)	Enrichment
Crude	120	31.1	0.26	—	—
Disulfide exchange	3.2	17.5	5.5	56	21
Anion exchange	0.012	1.5	125.0	5	481

Starting material was 20 pineal glands obtained from sheep killed at night. Purification was as described in the text. The values for specific activity, yield, and enrichment at different stages of purification for a typical run are given. The enzyme activity in the purest ion-exchange fraction (31) only was used in the calculation of final recovery and purity. Total recovery of activity from this step is about 40% and the overall recovery of activity from the procedure is about 20%.

Sepharose-cystamine followed by size exclusion using Sephacryl S-200, and finally affinity chromatography using Sepharose CoA. This procedure had two main drawbacks. First, it was slow, requiring 5–7 days for a complete run. Second, the chromatography columns had to be equilibrated with buffer containing BSA to stabilize the enzyme activity. As a result, the final preparation contained a predominant amount of BSA, and no estimate of the specific activity could be made; and, for the same reason $NaDodSO_4$-PAGE pattern was a reflection of the BSA. These problems are absent in the present procedure. Use of a pH change to elute the enzyme from Sepharose-cystamine column and a rapid anion-exchange chromatography step makes the present procedure short and obviates the need to use BSA to keep the enzyme active. As a result, meaningful estimates of specific activity of the final preparation can be made, and a sufficient amount of enzyme can be analyzed by $NaDodSO_4$-PAGE for characterization. Also, there is no problem of overloading the polyacrylamide gel with BSA.

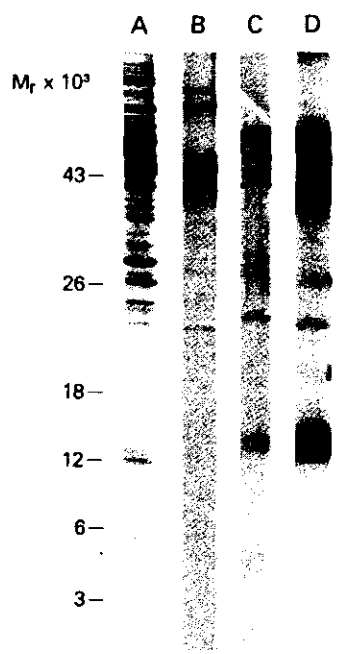

FIG. 2. $NaDodSO_4$-PAGE patterns at the different stages of purification. The samples were analyzed on a 15% acrylamide slab gel. Lane A, 10,000 *g* supernatant, 70 μg of protein, Commassie blue-R stain. Lane B, Sepharose-cystamine column fractions, 15 μg of protein, Commassie Blue-R stain. Lane C, MonoQ fraction of highest purity, 3 μg of protein, silver stain. Lane D, MonoQ fractions of highest purity labeled with ^{125}I-Bolton-Hunter reagent, 3 μg of protein, autoradiograms. The molecular mass standards are: ovalbumin, 43,000 daltons; α-chymotrypsin, 25,700 daltons; β-lactoglobulin, 18,400 daltons; cytochrome *c*, 12,000 daltons; bovine trypsin inhibitor, 6,200 daltons; insulin α–β chains, 3,000 daltons.

It should be mentioned that disulfide-exchange chromatography, the first step in the present procedure, is not significantly different from that used in the earlier procedure. The only difference is in the use of an increase in pH (6.5 to 8.5) for elution, in place of the elution with ATP (or other salts) used in the earlier procedure. One advantage of this modification is that the enzyme preparation obtained can be used for the second step (anion-exchange chromatography) without dialysis to remove the ATP or excess salts. In addition, some increase in the purification (21-fold versus 6-fold) is also obtained as a result of this modification. Despite this modification, the disulfide-exchange chromatography using Sepharose-cystamine is not a highly efficient purification procedure. Other proteins having accessible sulfhydryl groups bind to the Sepharose-cystamine column under the conditions of loading. We have been able to improve the purification using a change in pH at the elution stage. It may be possible to improve the efficiency of this purification technique further by using highly specific disulfide ligands instead of cystamine. Based on our observation that peptides containing disulfide groups can undergo disulfide exchange with enzymes with a high degree of specificity, we would predict that disulfide-exchange chromatography using specific disulfide-containing peptides as ligands may become more successful (Namboodiri et al., 1981, 1982).

An important question arises as to whether or not the enzyme we have purified is NAT, since the pineal

gland is known to contain both arylamine *N*-acetyltransferase and NAT (Voisin et al., 1984). The reason to think we have purified NAT and not arylamine *N*-acetyltransferase is that the latter enzyme is very unstable and complete loss of activity occurs during disulfide-exchange chromatography; in contrast 50–60% of NAT activity is recovered under this condition. Also, NAT is assayed using tryptamine, an arylalkylamine for which arylamine *N*-acetyltransferase has no detectable activity in pineal homogenates (Voisin et al., 1984).

In this procedure yield has been sacrificed for purity. The values for yield and fold purification in Table 2 refer to the purest ion-exchange fraction (fraction 31 in the present run). In a typical run, total recovery of activity from the ion-exchange step is about 40% and the final recovery in four fractions is about 20%. The material in three of these fractions is of significantly lower purity due to contamination by the major protein peak.

Even though we have been able to purify NAT about 500-fold, the unequivocal identification of the enzyme by NaDodSO$_4$-PAGE still remains unclear. This is because it is not possible to measure NAT activity of the protein bands. Accordingly, we can only speculate on the location of NAT on the NaDodSO$_4$-PAGE pattern, based on the known molecular weights of different forms of the enzyme. On the basis of the results of size exclusion chromatography using Sephacryl S-200, it appears that NAT can exist in three molecular forms depending on the ionic environment (Namboodiri and Klein, 1981). We have found that NAT is separated into two molecular forms ($M_r \simeq$ 100,000 and 8,000) on size-exclusion chromatography in the presence of ammonium acetate (0.1 *M*, pH 6.5) containing BSA (0.1 mg/ml) and DTT (10 m*M*). However, in the presence of sodium citrate (0.1 *M*, pH 6.5) containing BSA (0.1 mg/ml) and DTT (10 m*M*), NAT activity is detected in a single form of intermediate molecular weight ($M_r \simeq$ 30,000). In an earlier report Morrissey et al. have shown that rat pineal NAT in 105,000 *g* supernatant fraction can be separated into two molecular forms ($M_r \simeq$ 39,000 and 10,000) on Sephadex G-100 chromatography in the presence of potassium phosphate (50 m*M*, pH 6.5) containing β-mercaptoethylamine (4 m*M*) (Morrissey et al., 1977). Our observations show that the multiple forms of NAT depend on the ionic environment whereas the results of Morrissey et al. seem to indicate that interaction between sulfhydryl groups is involved in this process. Although further investigations are required to resolve this controversy, both sets of results indicate that molecules of NAT may exist within the pineal cells in polymeric forms. Based on these observations, it appears that the lowest molecular weight of the enzyme is in the 8,000–10,000 range. Since the molecular weight data obtained from size-exclusion chromatography are less accurate than those obtained from NaDodSO$_4$-PAGE, it is reasonable to suspect that the band observed at 12,000 M_r on NaDodSO$_4$-PAGE in the present study is NAT. Our observation that this protein band is prominent in the fraction containing NAT activity is consistent with this possibility. It is not clear if any other band or bands visualized by PAGE contain NAT activity. These questions may be answered when antibodies against NAT become available.

REFERENCES

Axelrod J. and Zatz M. (1977) The β-adrenergic receptor and the regulation of circadian rhythms in the pineal gland, in *Biochemical Actions of Hormones, Vol. 4* (Litwack G., ed), pp. 249–268. Academic Press, New York.

Binkley S., Klein D. C., and Weller J. L. (1976) Pineal serotonin *N*-acetyltransferase activity: protection of stimulated activity by acetyl-CoA and related compounds. *J. Neurochem.* **26,** 51–55.

Bolton A. E. and Hunter W. M. (1973) The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem. J.* **133,** 529–539.

Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins using the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Chibata I., Tosa T., and Matuo Y. (1974) Coenzyme A, in *Methods in Enzymology, Vol. 34* (Jakoby W. B., ed), pp. 267–271. Academic Press, New York.

Deguchi T. and Axelrod J. (1972) Sensitive assay for serotonin *N*-acetyltransferase activity in rat pineal. *Anal. Biochem.* **50,** 174–179.

Klein D. C. (1978) The pineal gland: a model of neuroendocrine regulation, in *The Hypothalamus* (Reichlin S., Baldessarini R. J., and Martin J. B., eds), pp. 303–327. Raven Press, New York.

Klein D. C. and Weller J. L. (1972) Rapid light induced decrease in pineal serotonin *N*-acetyltransferase activity. *Science* **177,** 532–533.

Klein D. C., Auerbach D. A., Namboodiri M. A. A., and Wheler G. H. T. (1981) Indoleamine metabolism in the mammalian pineal gland, in *The Pineal Gland: Anatomy and Biochemistry, Vol. 1* (Reiter R. J., ed), pp. 199–227. CRC Press, Boca Raton, Florida.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Morrissey J. H. (1981) Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Anal. Biochem.* **117,** 307–310.

Morrissey J. J., Edwards S. B., and Loverbeng W. (1977) Comparison of rat pineal gland and rat liver serotonin *N*-acetyltransferase. *Biochem. Biophys. Res. Commun.* **77,** 118–123.

Namboodiri M. A. A. and Klein D. C. (1981) Purification of ovine pineal N-acetyltransferase, in *Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects* (Usdin E., Weiner N., and Yodim M. B. H., eds), pp. 701–710. MacMillan Publishers, London.

Namboodiri M. A. A., Weller J. L., and Klein D. C. (1980) Evidence for inactivation of rat pineal serotonin *N*-acetyltransferase by protein thiol:disulfide exchange. *J. Biol. Chem.* **255,** 6032–6035.

Namboodiri M. A. A., Favilla J. T., and Klein D. C. (1981) Pineal *N*-acetyltransferase is inactivated by disulfide containing peptides: insulin is the most potent. *Science* **213,** 571–573.

Namboodiri M. A. A., Favilla J. T., and Klein D. C. (1982) Activation of acetyl CoA hydrolase by disulfide containing peptides. *J. Biol. Chem.* **257,** 10030–10032.

Namboodiri M. A. A., Sugden D., Klein D. C., Grady R., and Mefford I. N. (1985*a*) Rapid nocturnal increase in ovine pineal *N*-acetyltransferase activity and melatonin synthesis: effects of cycloheximide. *J. Neurochem.* **45**, 832–835.

Namboodiri M. A. A., Sugden D., Klein D. C., Tamarkin L., Grady R., and Mefford I. N. (1985*b*) Serum melatonin and pineal indoleamine metabolism in a species with a small day/night *N*-acetyltransferase rhythm. *Comp. Biochem. Physiol.* **80B**, 731–736.

Parfitt A., Weller J. L., Sakai K. K., Marks B. H., and Klein D. C. (1975) Blockade by ouabain or elevated potassium ion concentrations of the adrenergic and adenosine 3′,5′-monophosphate-induced stimulation of pineal serotonin *N*-acetyltransferase activity. *Mol. Pharmacol.* **11**, 241–255.

Reppert S. M. and Klein D. C. (1980) Mammalian pineal gland: basic and clinical aspects, in *The Endocrine Functions of the Brain* (Motta M., ed), pp. 327–371. Raven Press, New York.

Romero J. A., Zatz M., and Axelrod J. (1975) Beta-adrenergic stimulation of pineal *N*-acetyltransferase: adenosine 3′,5′-cyclic monophosphate stimulates both RNA and protein synthesis. *Proc. Natl. Acad. Sci. USA* **72**, 2107–2111.

Sarkar N. H. and Dion A. S. (1975) Polypeptides of the mouse mammary tumor virus. Characterization of two group specific antigens. *Virology* **64**, 471–491.

Voisin P., Namboodiri M. A. A., and Klein D. C. (1984) Arylamine *N*-acetyltransferase and arylalkylamine *N*-acetyltransferase in the mammalian pineal gland. *J. Biol. Chem.* **259**, 10913–10918.

Weissbach H., Redfield B. G., and Axelrod J. (1960) Biosynthesis of melatonin: enzymic conversion of serotonin to *N*-acetylserotonin. *Biochim. Biophys. Acta* **43**, 352–353.